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Volatile Components in Aqueous Essence and Fresh Fruit of *Cucumis melo* cv. Athena (Muskmelon) by GC-MS and GC-O

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A comparative study between the aromatic profile of muskmelon aqueous essence and the puree of fresh fruit was carried out using gas chromatography–mass spectrometry (GC-MS) and gas chromatography–olfactometry (GC-O). Results obtained show a total of 53 components quantified in the essence and 38 in the fresh fruit. In addition, four new components are described for the first time as contributors to the aromatic profile of muskmelon including 2-methyl-3-buten-2-ol, 2,3-butanediol, methyl 3-phenylpropionate, and ethyl 3-phenylpropionate (found only in the puree of the fruit). The olfactometric analysis revealed the presence of 25 components with aromatic activity. Esters, alcohols, and one sulfur component [ethyl 3-(methylthio)propionate] appear to be the most important contributors to the essence aroma. The aromagram of fresh fruit is richer in high molecular weight components, which have not yet been positively identified and do not present detectable peaks in the flame ionization detector.

Keywords: GC-MS; GC-O; muskmelon; aqueous essence; cantaloupe

INTRODUCTION

Currently seven well-known cultivars of *Cucumis melo* are cultivated in the United States, but members of the Reticulatus group (var. *reticulatus* Ser.) are probably the most commercially important melons. Muskmelon, netted melon, nutmeg melon, and Persian melon are all members of this variety. Approximately 100 compounds have been identified in the volatile fraction of muskmelon (1). Schieberle et al. (2), using aroma extract dilution analysis (AEDA), identified methyl 2-methylbutanoate, (Z)-3-hexenal, (E)-2-hexenal, and ethyl 2-methylpropionate as the primary odorants.

However, the characteristic aroma components of *C. melo* were previously identified as sulfur compounds including methyl (methylthio)acetate, ethyl (methylthio)acetate, 3-(methylthio)propanitrile, 3-(methylthio)propanol, 2-(methylthio)ethanol acetate, methyl (methylthio)propionate, ethyl (methylthio)propionate, and 3-(methylthio)propanol acetate (3). The importance of these sulfur compounds in the aroma of melons has been studied by different investigators (4–6). Wyllie et al. (6) reported additional sulfur volatiles in muskmelon (a variety of *C. melo*) and investigated the sensory significance of these components by gas chromatography–olfactometry (GC-O) and AEDA. Seven components were identified as significant odorants at the highest dilution, including ethyl 2-methylpropionate, S-methyl thiobutanoate, 3-(methylthio)propanal, 2,3-butanediol diacetate, 3-(methylthio)propyl acetate, dimethyl tetrasulfide, and one unknown component. Of these odorants only two contributed classical fruit notes, 2-methylpropionate and 3-(methylthio)propyl acetate. However, it was established that an increase in the concentration of the

extracts magnified the olfactory impact of the esters ethyl 2-methylbutanoate, methyl 2-methylbutanoate, and 2-methylbutyl acetate. These components presumably enhance the fruity characters of the melons.

The volatile components present in honeydew melon, a variety of *C. melo*, were studied by Buttery et al. (7). They identified (Z)-3-nonenyl acetate, (Z,Z)-3,6-nonadienyl acetate, (Z)-3-nonenyl acetate, 3-methyl-2-butenyl acetate, and ethyl 2-(methylthio)acetate for the first time. Later, Homatidou et al. (5) also reported the volatile components of *C. melo* cv. Cantaloupensis. These authors identified 105 components, among them 9 sulfur compounds, which contributed to the aroma. Of these, 2-(methylthio)ethanol was reported for the first time. The occurrence of six thioether esters, methyl (methylthio)acetate, ethyl (methylthio)acetate, 2-(methylthio)ethyl acetate, methyl 3-(methylthio)propionate, and 3-(methylthio)propyl acetate, in the aroma profiles of *C. melo* fruit was reported by Wyllie and Leach (4). They concluded that their presence and concentration appeared to be under genetic control, and some of them have been shown to have odor values, which indicate that they contribute to the overall aroma perception of the ripe fruit.

The Osme method is a widely used technique in GC-O analysis (8). Although this method does not apply a dilution factor to the sample, it does provide intensity information about the active aromatic components. From this point of view, the application of the Osme analysis to the muskmelon samples can give an idea about the principal odorants that contribute to the desirable aroma of muskmelon. The aim of the present work is to study and compare the aromatic profile of commercially available aqueous essence and a fruit puree of muskmelon, using gas chromatography–mass spectrometry (GC-MS) followed by GC-O analysis to determine the most important active contributors to the delicious aroma of this fruit.

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MATERIALS AND METHODS

Muskmelon Samples (*C. melo* cv. *Athena*). Unpasteurized muskmelon puree and aqueous essence were obtained from a local manufacturer of aqueous essences located in Florida. The samples were taken to the laboratory within 2 h after the industrial processing and frozen at -20°C for 2 months. Both the essence and the puree were derived from the same fruit source. The aqueous essence was obtained by a proprietary process, which utilizes low-temperature short-time steam distillation.

Extraction of Volatile Compounds. Muskmelon Fruit. Isolation of volatile compounds was made using a liquid extraction technique. Cyclohexanone (10 ppm) was added to 120 g of puree. The volatile components in the puree were then extracted with 80 mL of methylene chloride (Sigma Chemical Co., St. Louis, MO) by stirring for 1 h with a magnetic stirrer at $\sim 2^{\circ}\text{C}$. The resulting mixture was centrifuged for 10 min (5000 rpm, $\sim 3000g$), and the organic layer was dried with sodium sulfate and concentrated to ~ 1 mL using a distillation–rectification system, followed by further concentration to 0.1 mL by a flow of nitrogen.

Muskmelon Essence. Volatile components present in 3 mL of essence, with cyclohexanone at 10 ppm as internal standard, were extracted twice using 2 mL of methylene chloride for each extraction and mixed between two glass syringes connected by a stainless steel luer lock adaptor and combined. The final emulsion was centrifuged as above, and the organic layer was recovered. This fraction was concentrated to 0.1 mL using a flow of nitrogen. The extraction efficiencies for the muskmelon essence and muskmelon puree were 98%.

GC-MS Analysis. The qualitative and quantitative analyses of the volatile compounds were made using an Agilent model 5973N MSD mass spectrometer (MS) with a 7683 autosampler and a model 6890 gas chromatograph (GC) equipped with a $30\text{ m} \times 0.25\text{ }\mu\text{m}$ i.d. HP-5 (cross-linked phenylmethyl siloxane) column with $0.25\text{ }\mu\text{m}$ i.d. film thickness (Agilent, Palo Alto, CA). The initial oven temperature was held at 40°C for 6 min. It was then increased at $2.5^{\circ}\text{C}/\text{min}$ to 150°C and finally at $90^{\circ}\text{C}/\text{min}$ to 250°C . The injection port and ionizing source were kept at 250 and 280°C , respectively. The split ratio was 10:1 with $2\text{ }\mu\text{L}$ of sample injected. There was a solvent delay of 2 min, after which time the mass spectrum was collected from m/z 35 to 300, generating 5.27 scans/s. Compound identifications were made by comparison of the mass spectra and retention times with those of a corresponding reference standard (Aldrich Chemical Co., St. Louis, MO; Bedoukian Research, Inc., Danbury, CT) for all compounds except 2-methyl-3-buten-2-ol, propyl propionate, isobutyl propionate, 2-methyl-1-butanol acetate, propyl butyrate, butyl propionate, isoamyl propionate, 3-(methylthio)-1-propanol, ethyl 3-(methylthio)propionate, (*Z*)-6-nonen-1-ol, (*Z*)-3-octen-1-ol acetate, and methyl 3-phenylpropionate, which were identified by the NIST98 library (NIST, Gaithersburg, MD).

Quantification. For the purpose of quantifying identified components, linear regression models were determined using standard dilution techniques with cyclohexanone as internal standard. Target ions were used in the identification and quantification of each component by the mass spectrometry system. Standard reference compounds were used in all cases if commercially available. For the quantification of the 12 compounds that were not available, linear regression of similar components was used. The substitutions were 2-methyl-3-buten-2-ol by 3-methyl-2-butenol, propyl propionate by propyl acetate, isobutyl propionate by isobutyl butyrate, 2-methyl-1-butanol acetate by isoamyl acetate, propyl butyrate by butyl butyrate, butyl propionate by butyl butyrate, isoamyl propionate by isoamyl butyrate, 3-(methylthio)-1-propanol by methyl 3-(methylthio)propionate, ethyl 3-(methylthio)propionate by methyl 3-(methylthio)propionate, (*Z*)-6-nonen-1-ol by (*Z*)-3-nonen-1-ol, (*Z*)-3-octen-1-ol acetate by (*Z*)-3-hexenol acetate, and methyl 3-phenylpropionate by ethyl 3-phenylpropionate.

GC-O. The analysis was carried out using a Hewlett-Packard 5890 series II plus chromatograph equipped with a $30\text{ m} \times 0.25\text{ }\mu\text{m}$ i.d. HP-5 (cross-linked phenylmethyl siloxane)

column with $0.25\text{ }\mu\text{m}$ i.d. film thickness (Palo Alto, CA) directly connected to a flame ionization detector and a sniffing port (Gerstel, Inc., Baltimore, MD). The injector and detector temperatures were maintained at 250 and 280°C , respectively. The transfer line to the GC-O sniffing port was held at 300°C . The volume of extract analyzed and oven program temperatures were the same as those described above for the GC-MS. Humidified air was added in the sniffing port at $100\text{ mL}/\text{min}$. Three panelists were used for the detection and verbal description of the odor active components identified in both extracts. The flame ionization detector (FID) was used to determine the retention times of the volatile components in both extracts. They were compared with the retention times measured in the olfactometry runs and compared to reference standard. Data were collected using the Turbochrom data system (version 6, PE Nelson, San Jose, CA).

RESULTS AND DISCUSSION

Volatile Components in Muskmelon Essence and Muskmelon Fruit. Volatile components present in the aqueous essence and in the fruit puree were identified and quantified by GC-MS, yielding a total of 53 and 38 compounds, respectively (Table 1).

The volatile compounds identified in the commercial aqueous essence consisted of a total of 31 esters, 16 alcohols, 4 sulfur compounds, 1 ketone, and 1 aldehyde. Isobutyl acetate was the component detected in greatest concentration followed by butyl acetate, 2-methyl-1-butanol acetate, and 2-methyl-1-butanol. In the paste of the fruit, 20 esters, 11 alcohols, 5 sulfur compounds, and 2 aldehydes were quantified. Benzyl alcohol, 2-phenylethyl acetate, 2-methylpropanol, (*Z*)-3-hexen-1-ol, and phenylethyl alcohol were the components quantified in greatest concentration.

Recently, Beaulieu and Grimm (9) reported a total of 139 compounds isolated by a solid phase microextraction (SPME) device in cantaloupe at various developmental stages. Among them 53 were reported for the first time in fresh cantaloupe. In the present work, 4 compounds that have not been previously reported as constituents of the volatile components of *C. melo* have been identified as 2-methyl-3-buten-2-ol, 2,3-butanediol, methyl 3-phenylpropionate, and ethyl 3-phenylpropionate (found only in the puree of the fruit). (*Z*)-6-Nonenal, previously identified by Kemp et al. (10) and long known to have a characteristic melon aroma, was not found in detectable amounts in this study.

So far, the varieties and cultivars investigated in melons (*C. melo*) have been shown to contain typical mixtures of esters dominated quantitatively by ethyl acetate, butyl acetate, 2-methylbutyl acetate, ethyl butanoate, and ethyl 2-methylbutanoate and a range of (*Z*)-9 unsaturated esters and alcohols, some of which have a characteristic melon odor (7). Many authors have remarked on the importance of sulfur compounds in the aroma of melons. Wyllie and Leach (4), who studied the aroma profile of Makdimon melon, indicated that methyl (methylthio)acetate, ethyl (methylthio)acetate, 2-(methylthio)ethyl acetate, methyl 3-(methylthio)propionate, and 3-(methylthio)propyl acetate have odor values which indicate that they contribute to the overall aroma perception of ripe fruit. Wyllie et al. (6) detected 20 sulfur compounds in *C. melo* cv. Makdimon (muskmelon) and noted that of the 7 peaks identified as significant odorants, 4 contain sulfur, that is, *S*-methyl thiobutanoate, 3-(methylthio)propanol, 3-(methylthio)propyl acetate, and dimethyl tetrasulfide. In the present

Table 1. Aromatic Profile in Aqueous Muskmelon Essence and Muskmelon Fruit

component	RT	concn ^a (ppm)	
		muskmelon essence	muskmelon fruit
ethyl acetate	2.11	nc	nd
2-methyl-1-propanol	2.18	15.88 ± 1.55	2.30 ± 0.06
methyl propionate	2.20	41.92 ± 3.40	nd
1-butanol	2.52	37.61 ± 3.21	0.42 ± 0.03
methyl isobutyrate	2.77	15.93 ± 1.39	nd
2-methyl-3-buten-2-ol	2.80	nd	0.79 ± 0.07
3-hydroxy-2-butanone	3.13	65.30 ± 5.30	nd
ethyl propionate	3.16	73.86 ± 4.20	0.45 ± 0.01
propyl acetate	3.21	42.51 ± 1.72	0.08 ± 0.01
methyl butyrate	3.36	20.56 ± 1.15	0.50 ± 0.01
3-methyl-1-butanol	3.67	2.98 ± 0.38	0.21 ± 0.03
2-methyl-1-butanol	3.75	120.03 ± 6.16	0.76 ± 0.07
ethyl isobutyrate	4.20	16.03 ± 0.44	0.13 ± 0.01
1-pentanol	4.53	4.04 ± 0.21	nd
isobutyl acetate	4.65	334.54 ± 13.83	0.54 ± 0.06
methyl 2-methylbutyrate	4.72	24.12 ± 0.87	0.34 ± 0.01
hexanal	5.42	nd	0.25 ± 0.07
ethyl butyrate	5.55	81.17 ± 3.18	0.44 ± 0.03
propyl propionate	5.92	4.35 ± 0.20	nd
butyl acetate	6.15	168.21 ± 8.02	0.38 ± 0.06
2,3-butanediol	6.73	nd	0.47 ± 0.14
2-(methylthio)ethanol	7.31	1.8 ± 0.07	1.97 ± 0.22
ethyl 2-methylbutyrate	7.78	27.72 ± 1.81	0.50 ± 0.03
(Z)-3-hexen-1-ol	8.24	48.50 ± 2.18	2.00 ± 0.18
isobutyl propionate	8.75	3.39 ± 0.12	nd
1-hexanol	9.05	25.22 ± 1.04	1.09 ± 0.00
isoamyl acetate	9.23	0.69 ± 0.19	nd
2-methylbutyl acetate	9.41	127.64 ± 3.88	0.33 ± 0.02
propyl butyrate	10.41	1.91 ± 0.05	nd
ethyl pentanoate	10.58	1.23 ± 0.09	nd
methyl 2-(methylthio)acetate	10.95	1.24 ± 0.06	0.75 ± 0.01
butyl propionate	11.04	1.97 ± 0.08	nd
pentyl acetate	11.39	8.79 ± 0.19	nd
isobutyl butyrate	13.82	2.37 ± 0.05	nd
benzaldehyde	13.90	1.78 ± 0.21	0.75 ± 0.01
isoamyl propionate	14.82	1.60 ± 0.10	nd
1-heptanol	15.02	1.89 ± 0.15	nd
ethyl (methylthio)acetate	15.47	10.81 ± 0.37	0.29 ± 0.11
butyl butyrate	16.31	0.92 ± 0.02	nd
3-(methylthio)-1-propanol	16.13	nd	1.25 ± 0.05
ethyl hexanoate	16.56	3.60 ± 0.47	0.05 ± 0.01
(Z)-3-hexen-1-ol acetate	16.99	73.35 ± 2.09	0.27 ± 0.02
hexyl acetate	17.43	32.66 ± 1.75	0.62 ± 0.04
eucalyptol	18.07	0.79 ± 0.16	tr
benzyl alcohol	19.11	2.02 ± 1.41	5.21 ± 0.60
(Z)-3-octen-1-ol	20.11	3.58 ± 0.89	nd
1-octanol	21.22	14.54 ± 0.95	nd
2,3-butanediol diacetate	21.32	0.85 ± 0.03	0.23 ± 0.01
ethyl 3-(methylthio)propionate	22.71	1.41 ± 0.1	1.08 ± 0.01
heptyl acetate	23.48	2.81 ± 0.22	nd
phenylethyl alcohol	23.71	1.04 ± 0.04	1.79 ± 0.28
(Z)-3-nonen-1-ol	26.03	2.15 ± 0.31	nd
benzyl acetate	26.44	13.99 ± 0.99	0.23 ± 0.02
(Z)-6-nonen-1-ol	27.17	3.34 ± 0.18	nd
(Z)-3-octen-1-ol acetate	28.41	0.98 ± 0.14	0.13 ± 0.01
octyl acetate	29.25	3.49 ± 0.19	0.93 ± 0.01
3-phenylpropanol	30.58	3.04 ± 0.02	3.10 ± 0.20
2-phenylethyl acetate	31.69	1.58 ± 0.12	0.25 ± 0.04
methyl 3-phenylpropionate	32.62	nd	0.47 ± 0.01
ethyl 3-phenylpropionate	36.60	nd	0.48 ± 0.01

^a nd, not detected; tr, traces; nc, not calculated.

study only 5 sulfur compounds have been quantified, and they correspond with 2-(methylthio)ethanol, methyl 2-(methylthio)acetate, ethyl (methylthio)acetate, 3-(methylthio)-1-propanol, and ethyl 3-(methylthio)propionate. To determine the role of these and the remaining components identified in the aromatic profile, GC-O analyses of both samples were conducted.

GC-O. The olfactometry analysis of the muskmelon commercial essence and its corresponding fresh fruit

paste yielded a total of 25 active aromatic components, which are shown in Table 2. Seven of these compounds were not identified by GC-MS and three of these do not present any detectable peak in the FID.

Esters and alcohols appear to be the most important contributors to the desirable aroma of this fruit, because in general they contribute floral, sweet, green, and melon-like aromas. It is important to highlight the presence of three components. One was detected in the essence at 28.48 min, and the other two were detected in the fresh fruit puree at 25.9 and 33.38 min. These three peaks do not present any detectable peaks in the FID. However, they contribute fruity, melon-like, cantaloupe-like, and fresh-smelling aromas. This observation illustrates the importance of GC-O in the analysis of aroma in food because it allows the detection of components that really define the aroma yet cannot be readily quantified as a part of the aromatic profile of the fruit. To identify all of the components perceived by the GC-O, tentative identifications according to the Kovats index, the aroma as compared to standards, and the retention time of these components were made. As a result of this, nerol, nonyl acetate, and benzyl butyrate were tentatively identified as contributors to the aromatic profile in this fruit.

Schieberle et al. (2) studied potent odorants in cucumbers and muskmelons by AEDA, and they concluded that volatile esters were responsible for the fruity notes in the aroma of muskmelon. AEDA indicated that methyl 2-methylbutanoate and ethyl 2-methylbutanoate were the most intense odorants in the ester fraction. These results are in agreement with those found in this study because these two esters were found to contribute to a fruity, sweet, and cantaloupe-like aroma. At the same time, these authors declared that the green notes in the aroma profile of muskmelon were mainly caused by (*E*)-2- and (*Z*)-3-hexenal. 1,8-Cineole and (*Z*)-1,5-octadien-3-one were other significant odorants; however, these components were absent in the aromatic profile defined in this study. 1,8-Cineole (eucalyptol) was quantified but does not appear as a contributor in the aromagram. The potent odorant (*Z*)-6-nonenal, previously identified in muskmelon by Kemp et al. (10) and known to have the typical melon aroma, was also absent in the aromagram of the fruit. Similarly, 3,6-nonadienol, which was reported as a significant contributor to muskmelon flavor by Kemp et al. (11), was not detected in the present study by GC-MS. The absence of these components in the aromagram of the *C. melo* variety studied in the present work can be explained on the basis that the presence and concentration of some components in the aroma profile of *C. melo* could be due to genetic control (4). Nevertheless, the influence of the genetic variety on the aromatic profile of *C. melo* was not the purpose of this study.

Five sulfur compounds have been identified in this study, but only two of them have been perceived as contributors in the aromagram. Methyl 2-(methylthio)acetate was perceived in both samples (commercial essence and fresh fruit), and ethyl 3-(methylthio)propionate was detected only in the essence. This last compound contributed a clean, fresh, and melon-like aroma.

Figures 1 and 2 are examples of two aromagrams, derived from commercial essence and fresh fruit, respectively. For these replications a total of nine components have been detected for one of the panelists in

Table 2. Descriptors of the Volatile Components Detected by GCO in Muskmelon Essence and Muskmelon Fruit Puree

component	RT	Kovats index	descriptors	no. of panelists detecting component	
				essence	fruit puree
ethanol	1.56	553	ethanol	3	
acetic acid	2.15	589	acid, rancid, buttery	3	
ethyl propionate	3.48	663	acid, butter (weak)	3	
ethyl isobutyrate	4.69	721	floral, fruity, sweet	3	3
pentanol	4.9	730	acid, pungent		1
methyl 2-methylbutyrate	5.28	746	artificial strawberry, fruity, sweet	3	
isobutyl acetate	5.35	749	floral		2
ethyl butyrate	6.26	785	sweet, fruity, candy	3	
ethyl 2-methylbutyrate	8.64	860	cantaloupe-like, floral, fruity, melon	3	3
(Z)-3-hexen-1-ol	8.99	869	herbal, green	2	
2-methyl-1-butanol acetate	10.34	901	fresh, vegetable, banana	2	
methyl 2-(methylthio)acetate	11.76	929	baked potato, pungent, rancid	2	3
(Z)-3-hexenol acetate	18.41	1016	green, herbal, banana	3	
benzyl alcohol	20.72	1041	floral, fruity		1
(Z)-3-octen-1-ol	21.29	1047	toasted nut, smoky, dusty	3	
ethyl 3-(methylthio)propionate	24.00	1078	clean, fresh, melon, green	2	
heptyl acetate	25.08	1091	clean, fresh, floral	2	
NPI ^a	25.79	1101	sharp, pungent, acid	2	
NPI	25.90 ^b	1102	floral, fruity (faint)		2
(Z)-3-nonen-1-ol	27.56	1126	green, pungent, floral	3	
NPI	28.48 ^b	1140	fruity, melon-like, cantaloupe-like, green	3	
benzene propanol	32.60	1213	floral, fresh, green		1
nerol ^c	33.38 ^b	1228	fresh, meant		1
nonyl acetate ^c	37.61	1314	rancid, medicinal, pungent		2
benzyl butyrate ^c	39.16	1346	cantaloupe-like		1

^a NPI, not positively identified. ^b Not detected by FID. ^c Tentative identification.

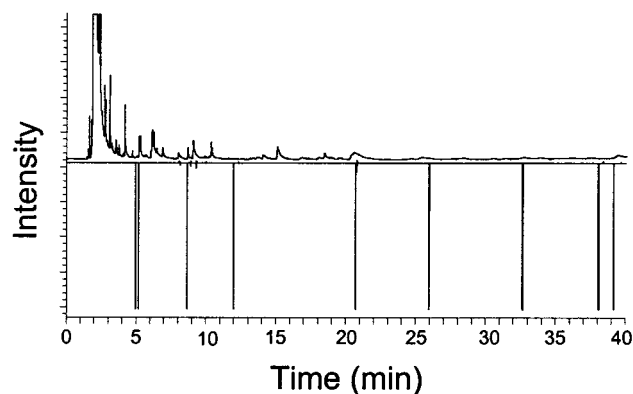


Figure 1. FID chromatogram (top) and GC-O aromagram (bottom) of a muskmelon extract from a puree that was used to create a fruit essence.

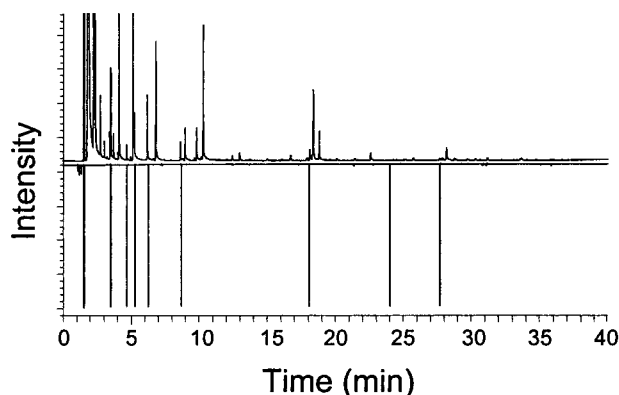


Figure 2. FID chromatogram (top) and GC-O aromagram (bottom) of a muskmelon extract from an essence that was created using the puree from Figure 1.

the essence and in the fruit paste. From these aromagrams, three principal differences are observed. First, the commercial essence is richer in low molecular weight esters. Second, high molecular weight components were

mainly perceived in the fruit paste. Finally, most of the compounds detected only in the paste do not show any detectable peak in the FID chromatogram. These results may suggest that the principal differences between the aroma of the essence and the aroma of the fruit puree are due to high molecular weight compounds, which are present in such low concentration that they cannot be detected by FID. However, they represent important aroma notes in the aroma of the fruit.

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